marked induction of RIC encoding gene, *ytfE* [2]. Subsequent studies have shown that: i) genes coding for RIC proteins are induced by nitrosative and oxidative stresses; ii) RICs contain a μ-oxo-bridged diiron centre of the histidine/carboxylate type [3]; and iii) deletion of *ric* renders bacterial strains more susceptible to reactive oxygen and nitrogen species and to macrophage killing. In the absence of RIC, bacteria display reduced endogenous activity of FeS-containing proteins, namely of the tricarboxylic acid cycle enzymes aconitase and fumarase, which became more prone to stress-induced damage in the *ric* defective strains; moreover, the activity of the damaged FeS enzymes could be restored upon exogenous addition of RIC [1, 4].

We have analysed the ability of *E. coli* RIC to assist the assembly of Fe-S centres in apo *Spinacia oleracea* ferredoxin and *E. coli* IscU. Using UV–visible, resonance Raman and EPR spectroscopies, we demonstrated that RIC participates in the synthesis of Fe–S centres in anaerobic reactions done in the presence of cysteine and cysteine desulfurase. The determined $Fe^{2+}$ and $Fe^{3+}$ iron dissociation constants revealed that $Fe^{2+}$ is more loosely bound than $Fe^{3+}$ with values that permit its mobilization *in vivo*. Finally, Mossbäuer spectroscopy showed that the iron atoms of the RIC diiron centre are more labile in the mixed-valence than in the diferric state. Altogether, we concluded that RIC is able to donate ferrous iron to the assembly of Fe-S centres [5].

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## S5.P7

### Making haem b in a different way

Susana L. Lobo[a], Andrew Lawrence[b], Célia V. Romão[a], Martin J. Warren[b], Miguel Teixeira[a], Lígia M. Saraiva[a]
[a]*Instituto de Tecnologia Quimica e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
[b]*Centre for Molecular Processing, School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK*
*E-mail address:* miguel@itqb.unl.pt

Until recently, modified tetrapyrroles such as haem, sirohaem or chlorophylls were believed to be synthesised along a unique branched biosynthetic pathway, the so-called classic pathway. However, our work in *Desulfovibrio* spp. revealed that haem *b* can be made by a distinct route, named the alternative pathway. In this newly discovered route, the alternative haem biosynthetic enzymes (Ahb) convert, in a series of unprecedented reactions, sirohaem into haem *b*. AhbA and AhbB firstly convert sirohaem to 12,18-didecarboxysirohaem, which is then transformed into iron-coproporphyrin III (Fe–Cp III) by AhbC. In the final step, Fe–Cp III is converted into haem *b* by AhbD. This alternative pathway may represent the ancestor of the classical one as it is active in old microorganisms such as the methanogenic Archaea [1].

To deepen our understanding of the alternative pathway, the final step promoted by *Desulfovibrio vulgaris* AhbD was extensively analysed. AhbD showed to be a haem *b* synthase that catalyzes the conversion of the two propionate side chains attached to C3 and C8 of Fe–Cp III into vinyl groups to form haem *b*, through promoting the cleavage of SAM methionine and the 5′-deoxyadenosyl radical. Electron paramagnetic resonance spectroscopy studies demonstrated that AhbD contains two $[4Fe–4S]^{2+/1+}$ centres which undergo conformational modifications upon binding of the substrates SAM and Fe–Cp III. Amino acid sequence comparisons indicate that *D. vulgaris* AhbD belongs to the radical SAM protein superfamily, with a GGE-like motif and two cysteine-rich sequences typical for ligation of SAM molecules and iron–sulfur clusters, respectively. Modelling studies allowed predicting a structural model of AhbD and proposing the putative pockets for the binding of the iron–sulfur centres and substrates [2].

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## S5.P8

### Involvement of SenC and Pcc1 in the assembly of cytocrome cbb3 oxidase from *Rhodobacter capsulatus*

Petru Iulian Trasnea[a], Marcel Utz[a], Fevzi Daldal[b], Hans-Georg Koch[c]
[a]*Institute for Biochemistry and Molecular Biology, Germany*
[b]*Department of Biology, University of Pennsylvania, USA*
[c]*Institute for Biochemistry and Molecular Biology, Stefan Meier Strasse, 79104 Freiburg, Germany*
*E-mail address:* petru-iulian.trasnea@biochemie.uni-freiburg.de

Cytochromes c oxidases (Cox) are crucial enzymes of the aerobic metabolism in eukaryotes and prokaryotes. The assembly and the activity of Cox require the incorporation of copper in the catalytic subunit I, where oxygen is reduced to water. How copper is inserted into Cox is unknown and was studied for cbb3-type Cox from the proteobacterium *Rhodobacter capsulatus*. This is a perfect model system because cbb3-type Cox is the only Cox present in this organism and because cbb3-type Cox contains Cu only in the catalytic subunit CcoN, while many other Cox contain an additional Cu center in subunit II. Several proteins haven been identified by genetic screens, which appear to play a so far uncharacterized role in the assembly of the CcoN Cu center. Among them are the two putative Cu binding proteins SenC and Pcc1. SenC is an integral membrane protein that contains a larger soluble domain, probably facing the periplasm. Pcc1, on the other hand, contains a cleavable signal sequence and is most likely secreted into the periplasm. Copper binding/transport activities and interaction of these proteins will be presented.

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## S5.P9

### In the absence of holo-COX, different supercomplex I+$III_2$ species are formed that differ in their structural components

Cristina Ugalde
*Fundación Investigación Hospital 12 Octubre, Spain*
*E-mail address:* cugalde@h12o.es

Mitochondrial respiratory chain complexes I, III and IV are associated in large molecular structures named supercomplexes (SC) or respirasomes. The functional roles of the respirasomes and intermediate supercomplexes, such as SC I + $III_2$ and $III_2$ + IV, remain to be fully determined. To address this issue, we used both metabolic labeling and a doxycycline-induced reversible inhibition of mitochondrial translation in mutant cybrids harboring homoplasmic mutations in the *COX1* and *COX2* genes, and we subsequently investigated the time-course incorporation of OXPHOS subunits into SC assembly intermediates. Our results suggest the stepwise formation of SC I + $III_2$ prior to the association of complex IV subunits. The identification of the structural components of this intermediate SC was initiated in human cybrids lacking complex IV, based on protein solubilization and separation by digitonin treatment and blue native electrophoresis (BN-PAGE) in combination with protein identification by high performance mass spectrometry (MS). This approach identified three bands corresponding to the so-called "supercomplex I + $III_2$" with apparent molecular masses around 1300 kDa on BN gels. The identities of these supercomplexes were determined on the basis of their subunit compositions and were validated by 2D-BN/SDS-PAGE. In the absence of COX1 and COX2, and hence holo-COX, we have identified respiratory chain supercomplexes composed of fully-assembled complexes I and III that also contained specific free COX subunits and novel supercomplex structural components that we have termed SCPs. The possible roles of these components on supercomplexes biogenesis will be further discussed.

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## S5.P10

### Involvement of CopZ in copper handling and delivery to cytochrome cbb3 oxidase

Marcel Utz[a], Petru-Iulian Trasnea[b], Fevzi Daldal[c], Hans-Georg Koch[d]
[a]*Institute for Biochemistry and Molecular Biology, Germany*
[b]*Institute of Biochemistry and Molecular Biology, University of Freiburg, Germany*
[c]*Department of Biology, University of Pennsylvania, USA*
[d]*Institute for Biochemistry and Molecular Biology, Stefan Meier Strasse, 79104 Freiburg, Germany*
*E-mail address:* utz.marcel@gmail.com

Heme-copper containing cytochrome oxidases (Cox) are highly conserved enzymes that terminate the electron transfer chain in aerobic and facultative anaerobic organisms. Although Cox are highly diverse in terms of subunit composition, cofactor content and oxygen affinity, they all share a conserved core subunit of 12 transmembrane domains, which harbors a low-spin heme group and a high spin heme-CuB binnucelar center. The insertion of CuB into the core subunit and the formation of the catalytic center is the least understood step in Cox assembly. cbb3-type cytochrome oxidases are perfect model enzymes for studying this essential process because the CuB center is the only Cu present in this type of enzyme, while the mitochondrial-like aa3-type cytochrome oxidases contain a second binuclear Cu center in subunit 2. We have identified several proteins that are involved in Cu binding and delivery to cbb3-type cytochrome oxidase in *Rhodobacter capsulatus*. Here we report on the characterization of the cytosolic Cu-binding chaperone CopZ. 64Cu binding assays and Atomic Absorption Spectroscopy assays indicate that Cu binding occurs via two conserved cysteine residues. Purified CopZ furthermore forms a redox-sensitive oligomer that is stabilized by the addition of Cu but destabilized by the addition of Ag. Experiments for identifying the interaction partner of CopZ and for determining its influence on cbb3-Cox activity will be presented.

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## S5.P11

### An exquisite chaperone for cytochrome c maturation: Kinetic characterization of the interactions between CcmI and different classes of apocytochromes c

Andreia Verissimo, Namita Schroff, Fevzi Daldal
*Department of Biology, University of Pennsylvania, USA*
*E-mail address:* andreiav@sas.upenn.edu

Cytochromes (cyts) *c* are ubiquitous electron transfer proteins involved in energy transduction in almost all living organisms. They have at least one heme binding site (CysXXCysHis) with a heme *b* cofactor (protoporphyrin IX-Fe) covalently bound via two thioether bonds. Although the presence and stereo-specificity of these bonds is universally conserved, cyts *c* are highly diverse in terms of their 3D structures, number of heme cofactors and redox properties. They can be grouped into 4 different classes based on their general fold and properties of their heme groups. *Rhodobacter capsulatus* produces efficiently several cyts *c* from different classes: class I cyts $c_2$, $c_y$, $c_1$, $c_o$ and $c_p$ used in respiration and photosynthesis; class II cyt $c'$ responsible for NO detoxification, and class III multihemic cyt DorC, involved in DMSO reduction.

Cyt *c* biogenesis is the post-translational process responsible for the covalent ligation of heme to the apocyt *c*. In α- and γ-proteobacteria, archaea, mitochondria of plants and red algae, the machinery dedicated to this process is designated Cyt *c* maturation (Ccm) — System I and involves nine membrane-associated proteins CcmABCDEFGHI. Of these, CcmI has a large periplasmic TPR containing domain that binds to the C-terminus of apocyt $c_2$. Besides being an apocyt *c* chaperone, CcmI forms the heme ligation complex with CcmFH and interacts with the heme handling protein CcmE and the thioredoxin CcmG.

We expressed and purified different soluble and membrane-bound apocyts (class I apocyts $c_2$ and $c_1$ and class II apocyt *c*') and studied the protein–protein interactions between CcmI and the apocyts by affinity co-purification. We used Bio-Layer Interferometry, a novel label-free analytical technique, to measure in real time molecular interactions between purified CcmI and its substrates. We found that CcmI recognizes apocyts *c* from different classes with remarkably different affinities ($K_D$). We also carried out a detailed characterization of the different apocyts in terms of their secondary structure by CD spectroscopy, and their ability to interact with heme *in vitro*. We will discuss our findings in the framework of a large Ccm maturase supercomplex, in which CcmI plays a pivotal role by trapping the apocyt *c* substrates while the other components act sequentially to ensure efficient heme ligation.

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